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Mass Spectral Imaging for Analysis of Pharmaceutical Ingredients

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Mass Spectral Imaging for Analysis of Pharmaceutical Ingredients

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ABSTRACT

Mass spectral imaging (MS-imaging) is a modern technique used for the analysis and characterization and distribution of pharmaceutical ingredients ,drugs,peptidies,lipids and other bioactive species in tissue section/ Tissue prints. Although now widely accepted, there remains a host of competing and complementary techniques including DESI, MALDI, SIMS, and DART ionization techniques that allow the researchers to obtain appreciable ion intensities for mapping purposes. Instrumentation further is, rapidly advancing with significant progress in TOF, ion trap, and quadrupole instrumentation for mapping. In this review, I will examine the current status and role of MS-imaging with emphasis on analysis of pharmaceutical ingredients

INTRODUCTION

In 1997, the concept of Mass Spectral Imaging (MSI) was first put forward by Caprioli. Over the past few years, this technique has suffered significant modifications. These changes have placed it on par with some of the otherwise conventional imaging procedures that are commonly known and that have been around for a very long time. The overall quality and spectrum of results that have been attained with this novel procedure is due to the technological advancements of this era. MIS, as the name suggests, has been developed on the basis of mass spectrometry. In this procedure, the images are created from the charts using a large number of spots that indicate the specific arrangement of many molecules. Generally, the three main procedures used for the process of ionization are secondary ion mass spectrometry (SIMS), matrix-assisted laser desorption ionization (MALDI) and desorption electrospray ionization (DESI). Mass Spectral Imaging can be used to distribute, analyse and characterize a series of compounds including lipids, drugs, peptides, pharmaceutical constituents and other materials present in samples of tissue. **(Caprioli et al., 1997), (Goodwin et al., 2012).**

Mass Spectral Imaging (MSI) comprises of two main parts, namely mapping and imaging. The mapping process refers to the manner in which the metabolites or compounds are distributed in any tissue sample. On the other hand, imaging can best be defined as the representation of the information obtained in the format of an image or picture. The picture created by imaging illustrates the quantity of ions that are present per position of mass. The mapping procedure involves the target of a specific region, of the tissue in question, with a ray of photons. Following this process, a time of flight mass spectrometer (TOF-MS) is employed to detect the emission of secondary ions from the targeted region of the tissue. The area in question is moved in a particular manner so as to be able to create a two dimensional map of the analytes under investigation.

The intensity of the signals emitted, can be gathered from a wide range of points on the tissue or specimen. Following this step, special software can then be employed to facilitate the transition from raw data to the image format. The use of MSI carries many benefits in comparison to many other existing procedures. Nevertheless, it lacks the ability, in this moment, to generate quantitative data for molecular diagnostic investigations. As a result of this backdrop, many investigators have started to centre their attention on developing a way to integrate both quantitative and distribution methods with the use of Mass Spectral Imaging. The main goal of this combination would be directed towards assessing the concentrations of molecules during the initial stages of pharmaceutical development.

Stoeckli et al. (2007) expresses the extent to which many factors must be taken into account to resolve this limitation of the MSI technique. According to these investigators, the use of the matrix-assisted laser desorption ionization (MALDI) method to assist with MSI quantification requires the understanding of certain basic factors and elements. In the first instance, the processes occurring within the specimen, as well as the ambience in which it is found, determine and condition the capacity to suppress ions in the same. The next issue of importance is that the matrix-assisted laser desorption ionization is characterized by allowing a specific yield of ionization per molecule. Another important element to consider is that the matrix deposition and its ability to extract, as well as its unique characteristics, determine whether or not the resulting sample signals are registered.

As illustrated in figure 1, the process of quantitative analysis in MSI involves three ways by which the procedure can be undertaken. These approaches include the following:

The utilization of a dilution range deposit on a control tissue section. The utilization of a range mixed with targeted tissue and then reconstituted: in this case, a matrix matched material is created and data about tissue-specific ion suppression can be attained. The utilization of an in – solution dilution range.

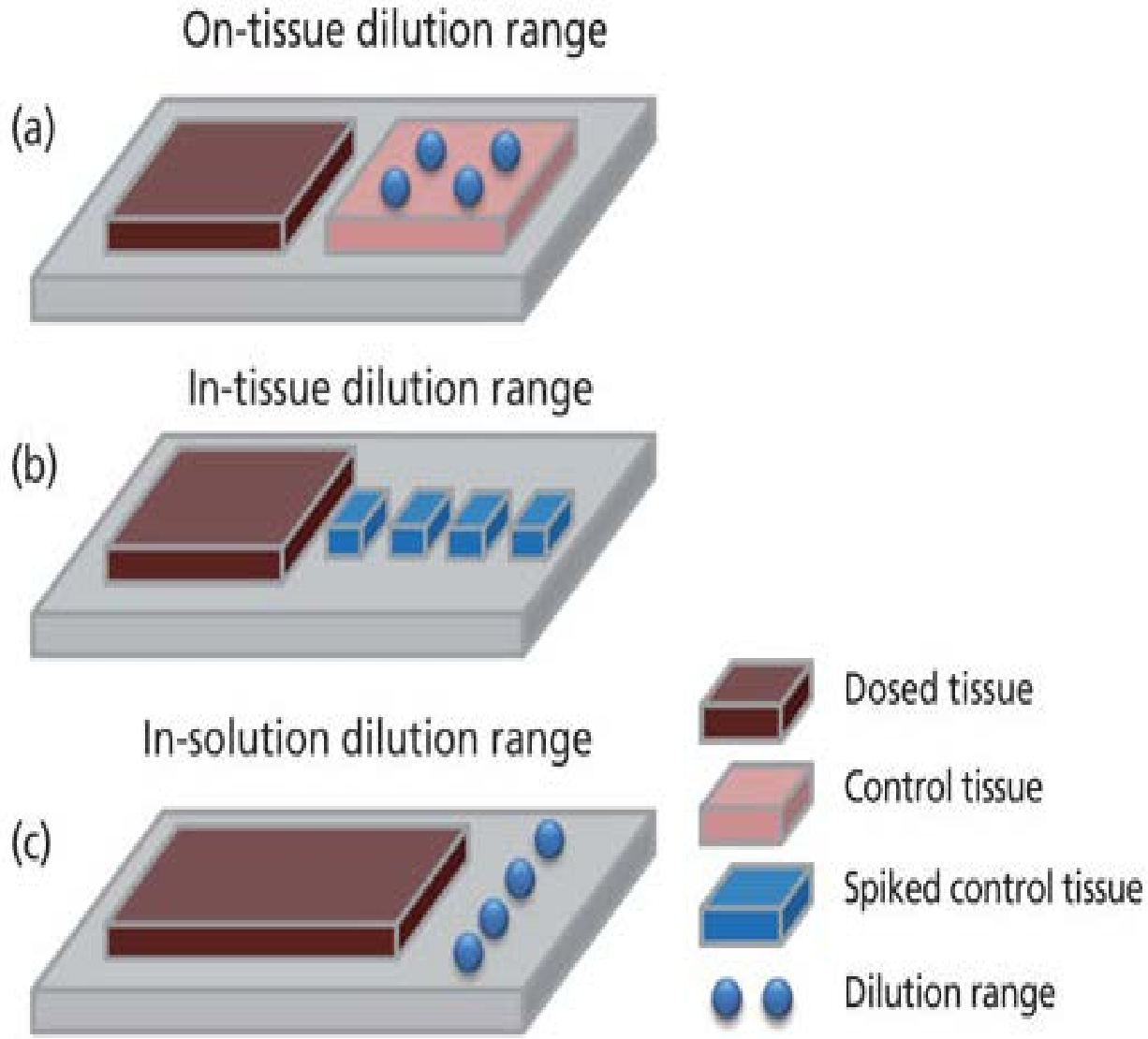


Figure 1. A summary of the three ways in which quantitative information can be attained using mass spectrometry imaging (MSI)

Sample Preparation

One of the most important aspects of the MIS process is that of preparing a standard and consistent sample. The first step in the preparation of the tissue involves the collection and conditioning of the sample. The specimen ranges from the use of organs all the way up to an entire organism. In the case of a quantitative analysis, standard models are utilized as a means of comparison with other methods. Following the gathering of the requisite sample, the specimen is sliced into the appropriate dimensions and placed on a slide. For this purpose, a cryomicrotome is utilized at low temperature ranges (-20 °C to -25 °C). The size of each specimen in question depends fundamentally upon the type of study to be undertaken. The sample is later arranged on a special slide coated with indium tin oxide or steel. The specimen can be pre- treated since pre-treatment enhances the overall quality of results. Unwanted sample impurities can be eliminated with the use of special solvents. The matrix deposition on the specimen constitutes one of the most relevant stages of the matrix-assisted laser desorption ionization imaging technique. **(Stoeckli et al., 2007).**

Emerging Technologies in Mass Spectrometry Imaging

i) MALDI Imaging: using this method, organic samples can be properly studied. In order to analyze the samples, the molecules in question are included into a special compound that aids in the processes of desorption and ionization whenever it is targeted with an ultraviolet laser. A mass spectrometer is then employed to evaluate the ratio of mass to charge of the ions in specific spots where ablation was carried out. Many different analytes are evaluated at the same time so as to generate a particular real time analysis of the molecules in the specific area of the tissue. **(Vanderbilt University Medical Centre, 2013).**

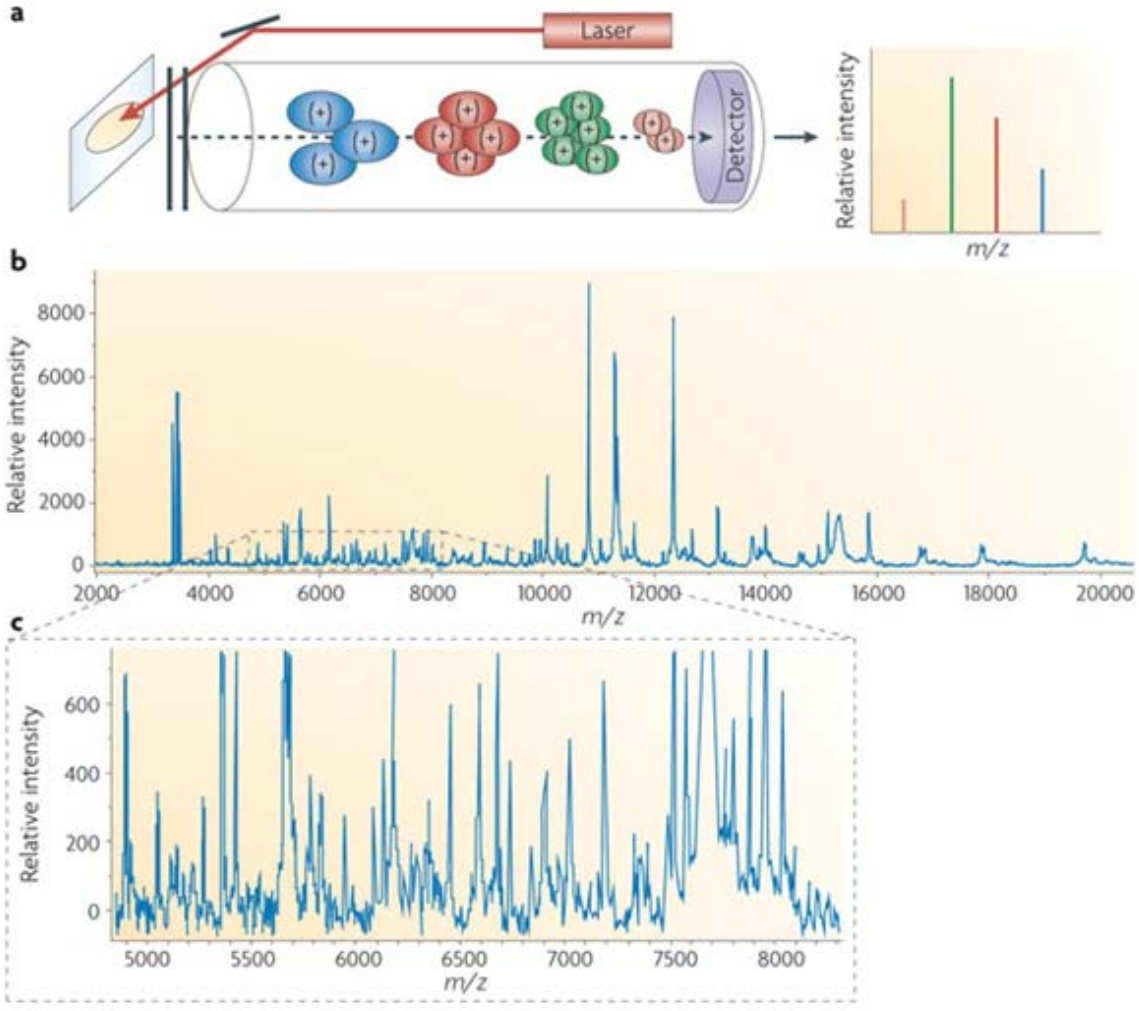


Figure 2: Principles of MALDI-TOF Mass Spectrometry.

Figure 2: Principles of MALDI-TOF Mass Spectrometry. a | Schematic outline of a typical separation of analytes in a linear matrix-assisted laser desorption ionization (MALDI)-time-of-flight (TOF) mass spectrometer based on their mass to charge ratio (m/z). b | Typical mass spectrum in the mass range between 2 and 20 kDa. c | A magnification of the mass range between 5 and 8 kDa.

The creation of a picture with the use of matrix-assisted-laser-desorption ionization mass spectrometry can be achieved by analysing the total region of the specimen in question. The specific pattern of laser movement facilitates the gathering of the requisite molecular data from a multiple spots that are arranged in a uniform order. The data present at each specific position is registered and graphically represented to demonstrate the association between the concentration of ions and their specific location. **(Vanderbilt University Medical Center, 2013).**

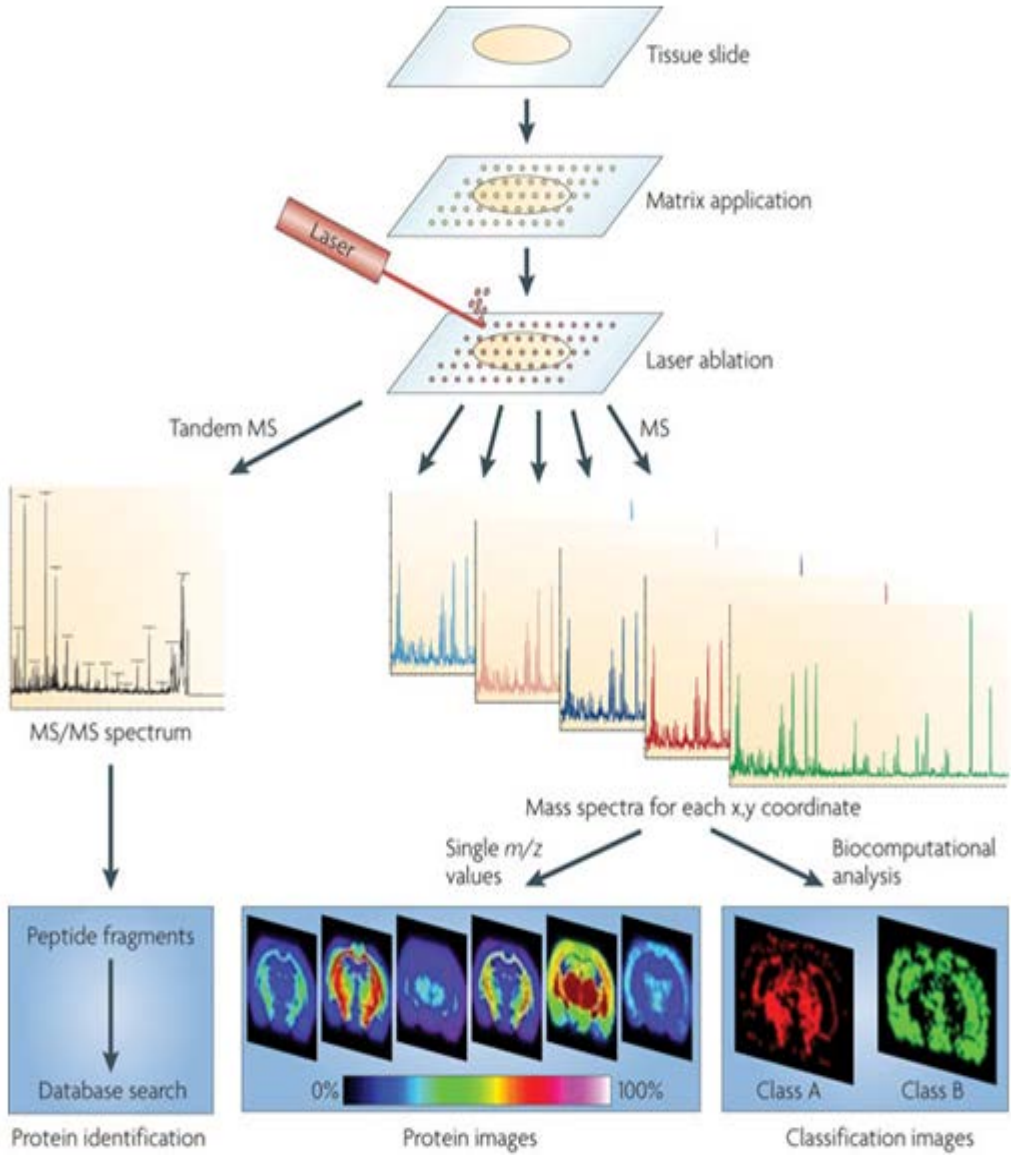


Figure 3: MALDI Imaging Mass Spectrometry.

Schematic outline of a typical workflow for fresh frozen tissue samples.

Given the efficiency and effectiveness of the results obtained with MALDI imaging mass spectrometry, it can be considered as an indispensable instrument for analysing the presence and location of certain drugs and their bi-products in samples. The benefits of using this tool are numerous and these include precision, lower cost and greater turnover of results, as compared to the traditional imaging tools. Pharmaceutical lines have employed this technique with favourable outcomes.

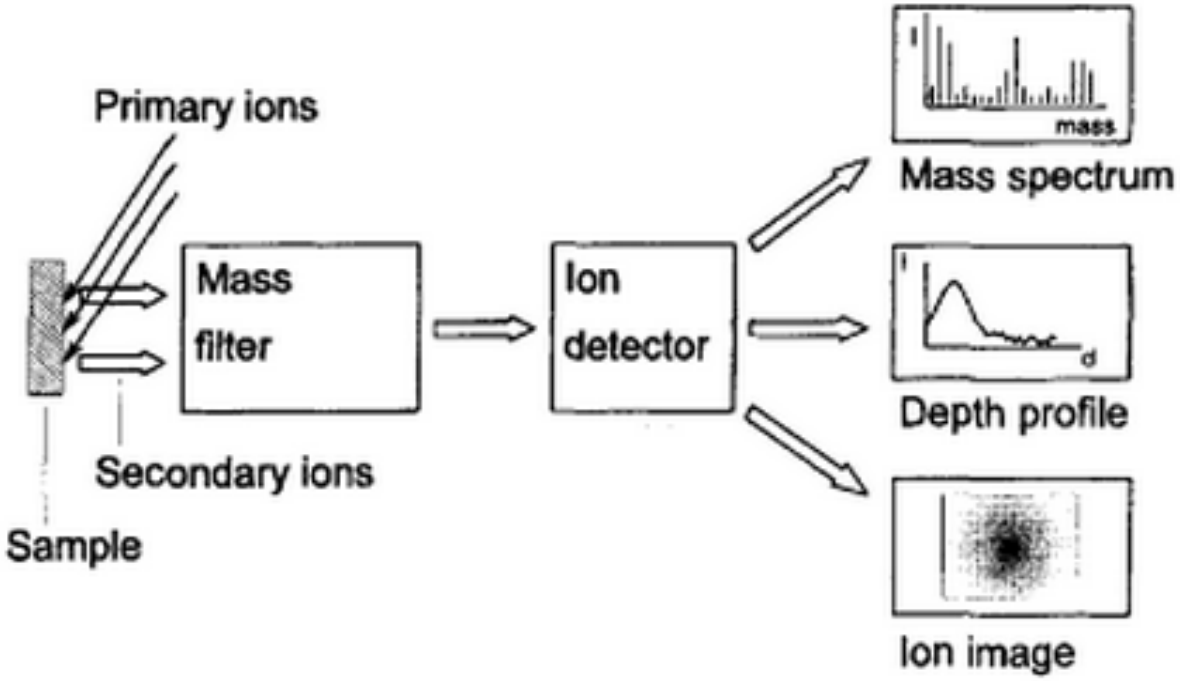
ii) Secondary ion mass spectrometry (SIMS) imaging: this technique is commonly utilized to analyse the chemical constituents of solid materials. The main idea of the technique is the bombardment of a sample with high energy ions in a vacuum sealed medium. The result of this is the release of secondary ions from the surface of the specimen under study. Following the release of secondary ions, they are then processed with a mass spectrometer where they are distinguished based upon their ratio of mass to charge. A detector is subsequently employed to obtain the results of the analytes. Secondary ion mass spectrometry is also referred to as a surface procedure because the secondary ions can only be dislodged from the outer layers of the specimen. Continuous bombarding can later lead to the dislodging of ions from deeper layers of the sample. The latter forms the basis for analysis using depth profiling. The capacities demonstrated by SIMS have been exploited in other areas such as the electronics field. **(Amelinckx et al., 1997)**.

Another novel function of the SIMS tool is the addition of the ability to graphically represent the results. The acquisition of the concentration of the secondary ions in terms of their point of emission aids in the mapping of the results. As a result of this mapping process a two dimensional surface composition can be obtained. A three dimensional composition can be achieved by merging the imaging capabilities of the process with the dept profiling. **(Amelinckx et al., 1997).**

The main elements of the SIMS imaging technique is illustrated in figure 4.

Figure 4: Schematic diagram of a secondary ion mass spectrometer set-up and the basic types of data acquisition: mass scan, depth profile and ion mapping.

SIMS is characterized by being a very sensitive procedure. Nevertheless, due to the bombardment procedure, organic compounds disintegrate and the data associated with their presence is destroyed. Ion images obtained in the process can be attained by two distinct forms. **(Amelinckx et al., 1997).**



The mass spectrometer is designed in such a way so as to ensure that the lateral arrangement of the secondary ions is maintained. This allows the precise detection of ions proceeding from their point of emission and reaching the detector designed to capture the specific orientation of the ions in space. The image that develops from the detector is due to the filtering of the masses of the various secondary ions. **(Amelinckx et al., 1997).**

Characteristics of SIMS

SIMS can be utilized for the detection of all of the elements making up the periodic table. The specific properties of the specimen, as well as the conditions under which the technique is carried out, would both affect the variability of the results and the overall sensitivity of the procedure. Once the working environment is conducive, sensitivity is remarkably enhanced. In comparison to other similar procedures, SIMS stands out for its capacity to detect a wide spectrum of elements and other compounds, with a high level of precision and sensitivity. The integration of imaging with depth profiling is specific to SIMS and aids in rebuilding the three dimensional distribution of elements in a sample. **(Amelinckx et al., 1997).**

Sample Chamber

A vacuum sealed environment is maintained within the sample chamber of the SIMS tool in order to keep the contamination levels at a minimum. A deficit of such environmental conditions would give rise to a continuous deposit of molecules on the surface of the specimen. The typical sample mounted unto a holder possesses a diameter of about 1 cm. The holder permits adequate sample movement and caters for sample cooling. **(Amelinckx et al., 1997).**

Mass Spectrometer

SIMS utilizes different versions of mass spectrometers. These include quadrupole mass filters, double-focusing magnetic mass filters, double-focusing magnetic sector instruments, and time-of-flight (TOF) mass analyzers. The quadrupole spectrometers permit a mass spectrum of up to 1000 amu; nevertheless, this type of spectrometer only allows unit mass resolution and has a very low efficiency of transmission.

Once imaging is required, their characteristics only permit their utilization in ion microprobes. In the case of magnetic sector mass spectrometer the mass spectrum is halved, only permitting approximately 500 amu. In this spectrometer, the efficiency of transmission shares and inversely proportionate relationship with the mass resolution. **(Amelinckx et al., 1997).**

Ion Detection and Image Registration

A particular sensitive tool is used to detect the secondary image in an ion microscope. A micro channel plate serves the function of a converter and amplifier of ions to electrons. This marks the initial phase of ion detection. The voltage applied to the system determines the gain of the micro plate channel. A resistive anode encoder or a charge – coupled device is employed to detect the electron cloud that is present. The electron cloud can also be transformed into photons with the use of a phosphor screen. Subsequently, a video camera picks up the image and the same is converted into a digital picture with the help of a frame grabber. **(Amelinckx et al., 1997)**

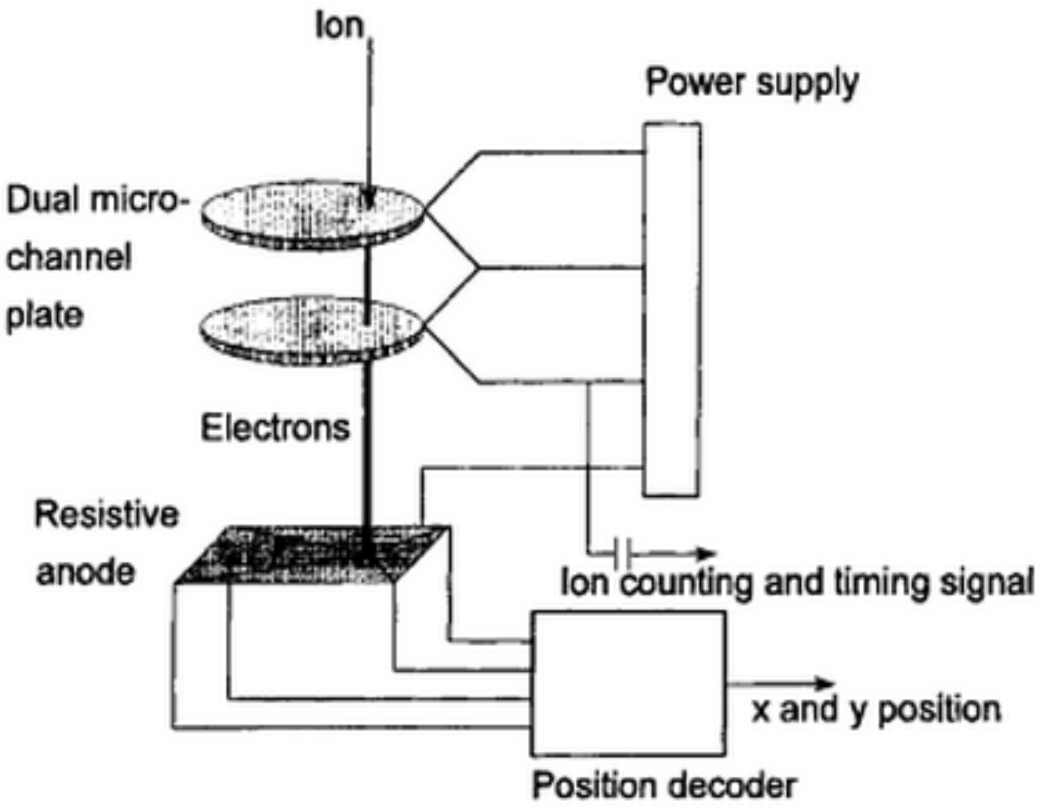


Figure 5: Schematic diagram of an RAE detector used as a position-sensitive ion counter in SIMS imaging

Interpreting and Processing of Ion Images

Under ideal circumstances, the variation in the concentration of ions should be the only cause of the contrast that forms on the ion image. Nevertheless, the discrepancies in contrast observed and the actual concentration of ions can occur due to spurious contrast pathways. The origin of spurious contrast includes: chromatic or energy contrast, orientation or crystallographic contrast, matrix contrast and topographic contrast. The undesirable variations of intensity in the image can also be attributed to the variable response of the position – sensitive ion detector.

The separation of concentration contrast from all other possible sources of contrast is vital for obtaining unambiguous results. **(Amelinckx et al., 1997)**

Analysis of Image Depth sequence

The inclusion of depth as a parameter in the images obtained gives rise to new options for processing data. The images associated with one type of ion in a sample can be superimposed in such a way so as to obtain a three dimensional idea of the intensity and location of the same. In the case of depth profiling, the intensity of the contrast can be obtained for every surface area of a particular stack and the total results can be superimposed to obtain a three dimensional orientation in space. **(Amelinckx et al., 1997)**

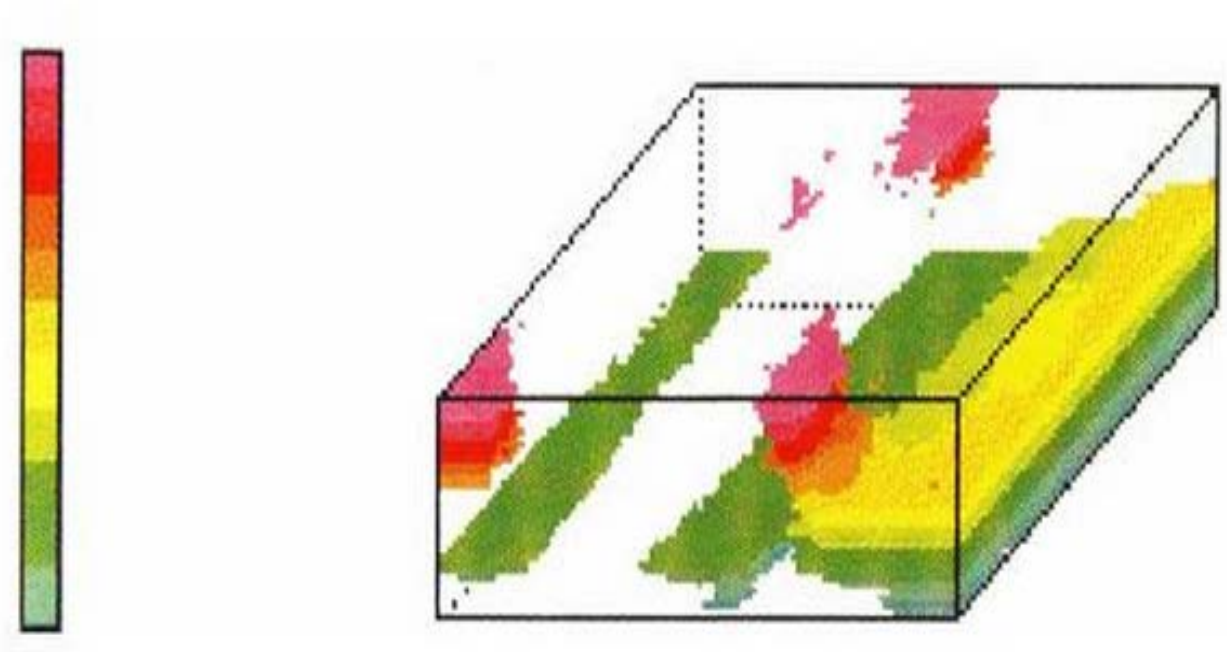


Figure 6: Reconstructed pseudo three-dimensional view of the aluminium distribution in the interior of a sample. In the transparent view the grey scale corresponds to depth in the sample.

Sample Requirements

The sample would naturally begin to conduct once it is bombarded with charged particles. This is mainly due to the fact that ions are dislodged from their original positions within the sample, thus causing instability. The build-up of charges can hamper the extraction of secondary ions. The use of a conducting layer to coat the sample is not beneficial during the SIMS technique. The issue can be resolved with insulating samples, by the use of a positive ion beam and counteracting the accumulation of charge with a flow of electrons on the sample surface. The texture of the surface affects the results obtained in SIMS. The angle of the primary beam determines the overall yield of secondary ions. The rougher the surface, the greater are the chances of unequal distribution of the effect of the primary beam. This process can lead to a topographic induced contrast on the final image. The roughness of the surface can also create ambiguity with regards to depth profiling since the secondary ions would not be dislodged from the same level at the same time. **(Amelinckx et al., 1997)**

iii) DESI Imaging: the process of desorption electrospray ionization involves the application of a solvent to a sample in the form of an electrospray and under controlled environmental conditions. This procedure is known for being highly sensitive. The ionized spray strikes the sample and causes desorption of ions in the sample. Subsequently, these desorbed ions are transferred to a mass spectrometer where detection occurs. This technique has been employed in areas involving protein and drug analysis, among other uses. **(Ashish, 2007).** The specimen is applied to a sample surface. A movable platform is used to mount the sample surface and it is adjusted to coincide with the capillary of the mass spectrometer. The electrospray capillary is directed towards the sample and the spray solvent is inserted into the inner capillary. The ion source is subsequently attached to a source of high voltage. **(Ashish, 2007).**

The outer section of the capillary serves the function of transporting the jet of nebulizer gas. This gas stabilizes the spray and carries the droplets. The sample spot receives the ionized spray and becomes ionized likewise. The droplets or desorbed ions are then transported through the capillary of the mass spectrometer and detection of the same occurs. The gathering and processing of the information can be achieved with the use of a computer. **(Ashish, 2007).**

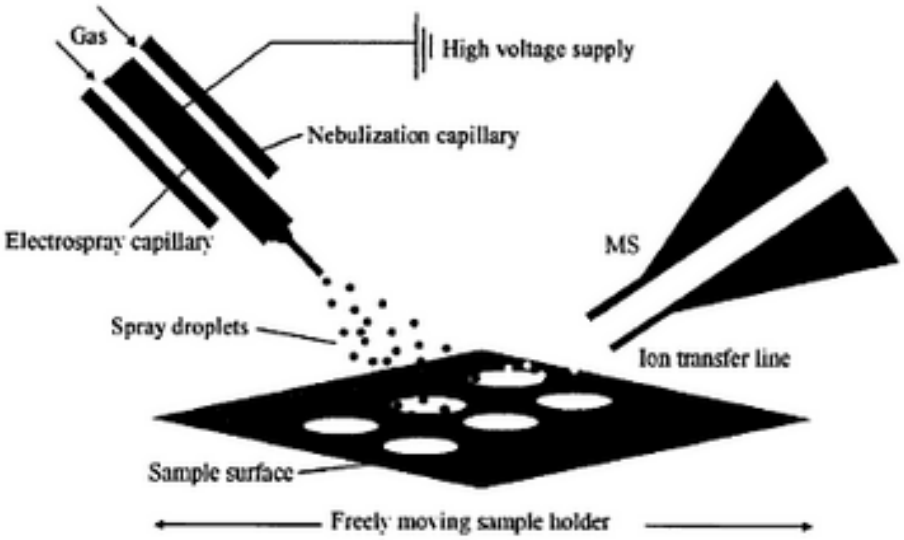


Figure 7. Schematic of a typical DESI-MS experimental setup

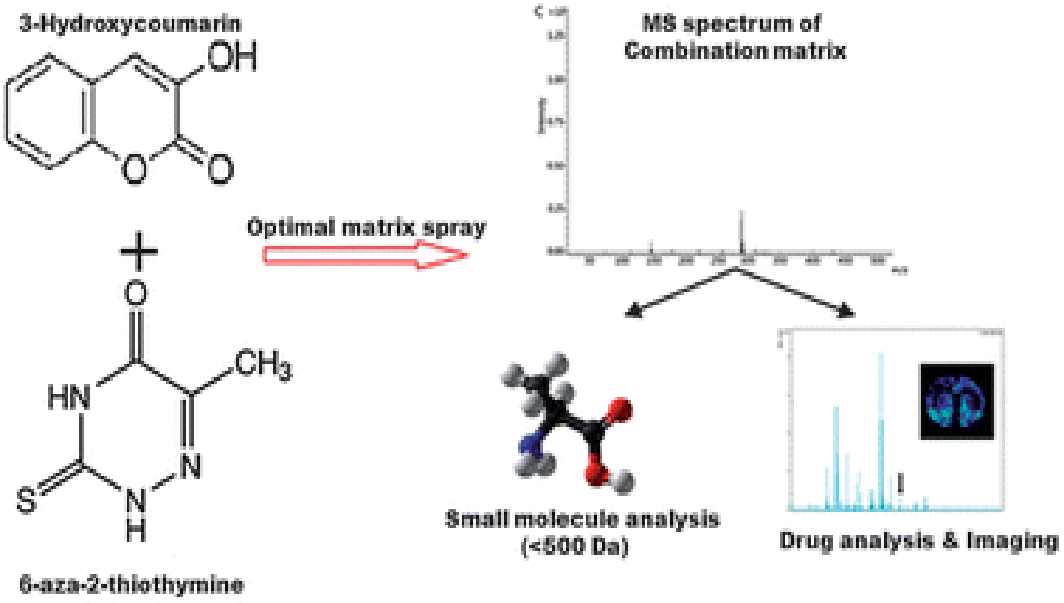
There are distinct ways in which ionization occurs for DESI. These are as follows: Splashing of ionized droplets from the specimen, Ion exchange between the sample and the incoming droplets associated with momentum transfer. Desorption of a neutral specimen from the sample surface and subsequent ionization in the charges plume of the electrospray. A combination of the above mentioned mechanisms. **(Ashish, 2007).**

Pharmaceutical Application of Imaging mass spectroscopy

DESI has been employed in the investigation of many pharmaceutical products without them being previously separated. Examples of these include ointments, tablets and liquids. Many investigators have carried out analysis using DESI. Chen et al. (2006), Weston et al. (2005) and Williams et al. (2005) have all employed the use of DESI for the analysis of tablets. Weston et al. (2005) have also worked in the analysis of ointments. Leuthold et al. (2006) and Rodriguez – Cruz (2006) focused their attention on the tablets that are used and abused as drugs. According to Chen et al. (2006), DESI guarantees high quality information and has the advantage of not bearing a carry-over effect. The importance and significance of DESI can be further exemplified with the fact that this method can quickly detect potentially hazardous and fake tablet preparations. There is a growing trend in the utilization of DESI for identifying the presence of certain metabolites in body fluids, such as urine. These metabolites can be early signal of a pathological process in the body. The requirements for the preparation of the samples are minimal and this facilitates ease of processing of hundreds of specimen at any given time. Chen et al. (2006) discovered over 80 metabolites in urine with the use of DESI.

Davies et al. (2004), utilized SIMS to analyze the chemical composition of the surface of a drug delivery system. The images obtained showed a uniform lateral distribution of the surface of the polymer. A project based on the analytical study of drugs using TOF-SIMS was developed in order to characterize and quantify ingredients in medicines. The feasibility of this method and outcome depends fundamentally on the understanding of the factors affecting the release and detection of secondary ions. **(“Characterization of Binary Drug by TOF-SIMS”, 2013)**

Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is commonly utilized to analyze compounds of high molecular weight. A classic example of analytes processed with this technique is proteins. To counteract the backdrops of MALDI, investigators have been experimenting with a huge spectrum of chemical compounds as matrices. These compounds aids in the ionization of small molecules in the mass spectrometer. Shanta et al. (2012) developed a particular matrix that was able to ionize even amino acids. The matrix can be utilized for imaging mass spectrometry procedures, given its peculiar characteristics.



Desorption electrospray ionization-mass spectrometry was assessed for the analysis of liquid specimen and the results obtained were significant.

The results showed that bovine serum albumin was ionized with the use of DESI thereby contrasting the belief that only solid samples with smaller molecular weight could be successfully utilized. The next important result was that DESI was successfully applied to the analysis of protein solutions without prior cleaning or separation via chromatography. Another important observation is bio fluids were successfully analysed without pre-treatment. The results points to the droplet pick up mechanism as being the reason behind the ionization of liquid samples. **(Miao et al., 2008).**

Miao et al. (2008) highlighted other advantages of DESI as compared to other similar techniques. These advantages include: Inhibition of electrochemical reduction in the negative ion mode is possible for liquid sample DESI,Reactive DESI can be carried out with ion/ion reactions of Zn(II) complexes for the selective binding of phosphoserine in the presence of serine,DESI experiment can also be performed directly to liquid samples flowing out of a pumped syringe needle tip, thereby facilitating rapid analysis of on-line coupling of electrochemical cell with DESI-MS is possible,DESI – MS can be extended to areas of forensic medicine and clinical laboratory. **(Miao et al., 2008).**

Limitations of Mass Spectral Imaging

The cost of running the analysis constitutes the fundamental limitation of MSI. To a lesser extent, the spectrum of detection of some metabolites or drugs may also pose a threat of limitation.

CONCLUSION

Mass spectral imaging has been observed to play a vital role in the analysis of metabolites, drugs and tissue samples. The analytes utilized in these studies possess varying degrees of success with the techniques utilized. Mass spectral imaging has the potential to become a key analytic tool in the investigation of compounds, tissues and other samples. Extension to other fields of study is possible since this technique can be utilized to characterize compounds studied in various disciplines.

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